Scientific Institute of Public Health

Relative potency estimates for PAHs by means of the CALUX assay

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Introduction

CALUX bioassay : binding and activation of the aryl hydrocarbon receptor (AhR) by dioxinlike compounds

- → **dioxins**
- → **PCBs**
- → **polycyclic aromatic hydrocarbons (PAHs)**

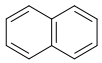
Polycyclic aromatic hydrocarbons (PAHs)

- > 100 compounds with 2 or more aromatic rings
- mutagenic and carcinogenic effects

- **EPA**: 16 priority PAHs

  → several other PAHs that are not routinely analyzed are responsible for mutagenic and carcinogenic effects. (e.g. ‘heavy PAHs’)

US EPA PAHs

16 US EPA priority PAHs	MW	Structure
naphtalene	128	
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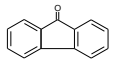
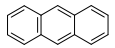
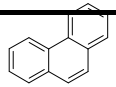
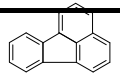
acenaphtene	154	
fluorene	166	
anthracene	178	
phenanthrene	178	
fluoranthene	202	
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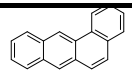
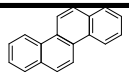
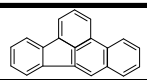
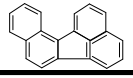
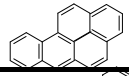
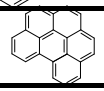
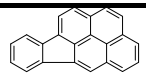
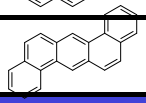
benzo(a)antracene	228	
chrysene	228	
benzo(b)fluoranthene	252	
benzo(k)fluoranthene	252	
benzo(a)pyrene	252	
Indeno(1,2,3-c,d)pyrene	276	
benzo(g,h,i)perylene	276	
dibenzo(a,h)antracene	278	

Polycyclic aromatic hydrocarbons (PAHs)

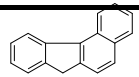
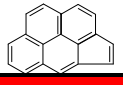
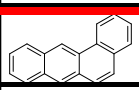
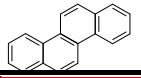
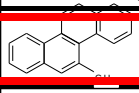
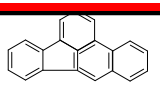
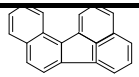
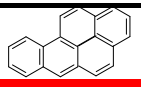
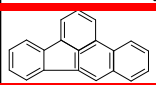
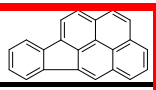
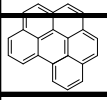
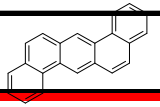
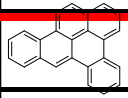
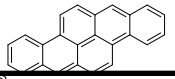
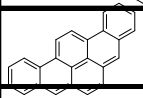
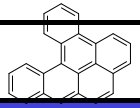
- > 100 compounds with 2 or more aromatic rings
- mutagenic and carcinogenic effects

- **US-EPA**: 16 priority PAHs

  → several other PAHs that are not routinely analyzed are responsible for mutagenic and carcinogenic effects. (e.g. 'heavy PAHs')

- **European Union (2002)**
  - list of 15 PAHs (+ BcF) that are of major concern for human health due to their toxic properties
  - monitor those 15 priority PAHs (+BcF) in food and the environment to enable long-term exposure assessments

EU priority PAHs

Name	MW	Structure
benzo(c)fluorene	216	
cyclopenta(c,d)pyrene	226	
benzo(a)antracene	28	
chrysene	228	
5-methylchrysene	242	
benzo(b)fluoranthene	252	
benzo(k)fluoranthene	252	
benzo(a)pyrene	252	
benzo(j)fluoranthene	252	
Indeno(1,2,3-c,d)pyrene	276	
benzo(g,h,i)perylene	276	
dibenzo(a,h)antracene	278	
dibenzo(a,e)pyrene	302	
dibenzo(a,h)pyrene	302	
dibenzo(a,i)pyrene	302	
dibenzo(a,l)pyrene	302	

CALUX bioassay

→ mouse H1L6.1 cell line from XDS

→ 24 h exposure time

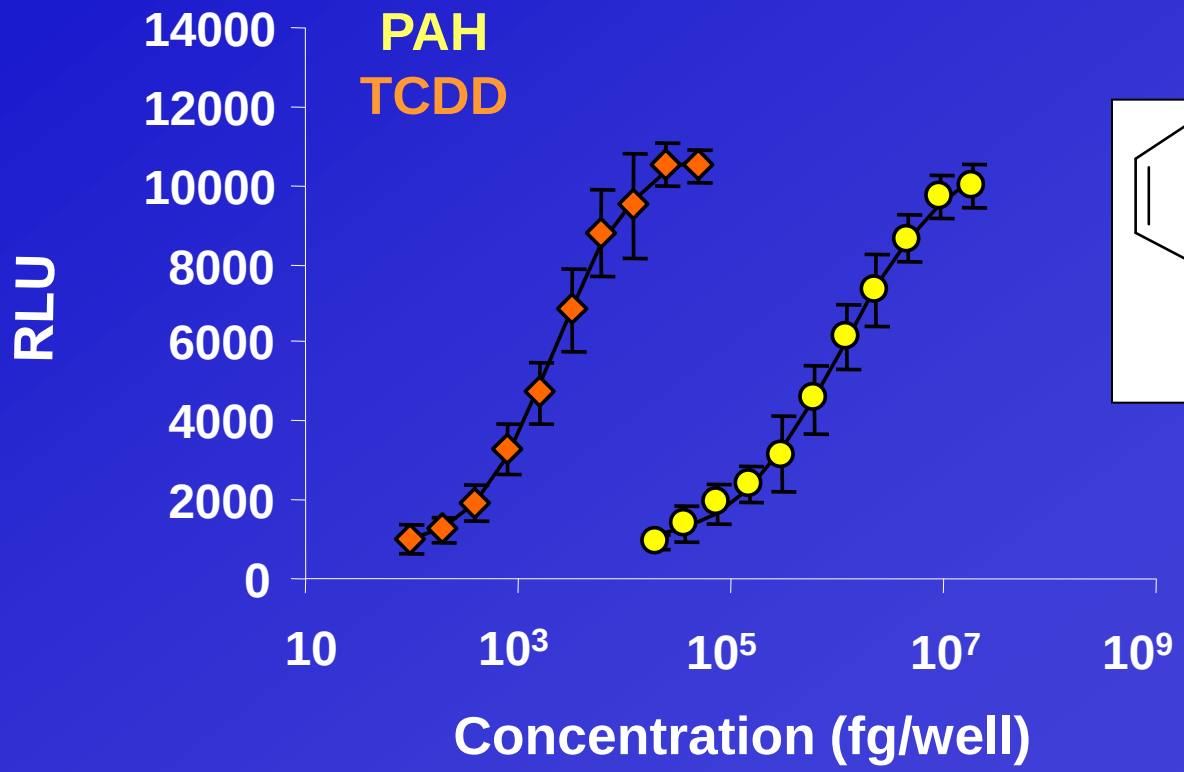
→ maximum concentrations tested in the *in vitro* bioassays:

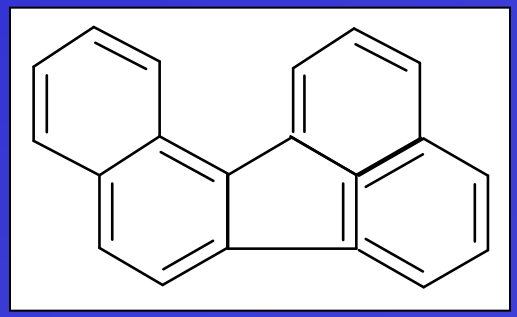
- 50-400 mg/L (in DMSO)

Dose response curves

- *Normal response*

Benzo(k)fluoranthene

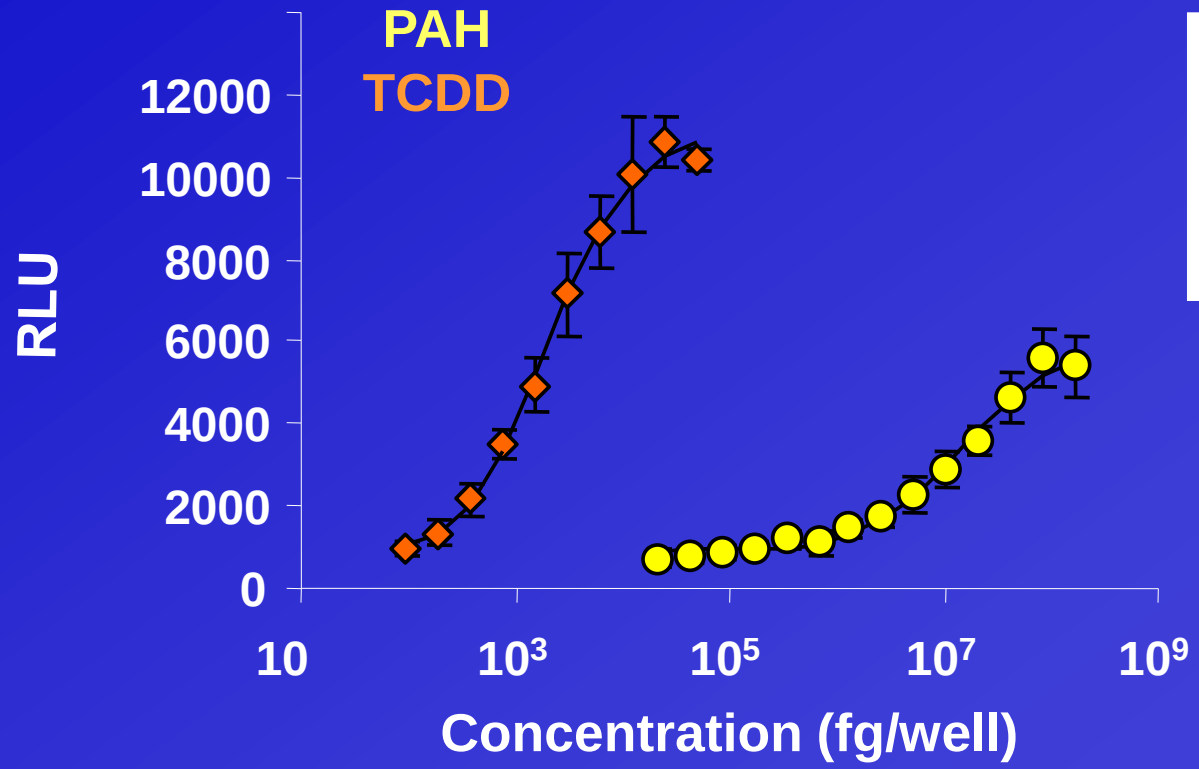




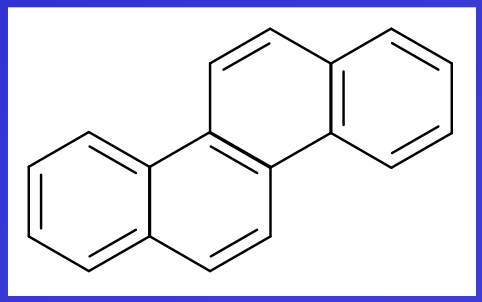
24 h exposure

Dose response curves

- *Low response*



Chrysene

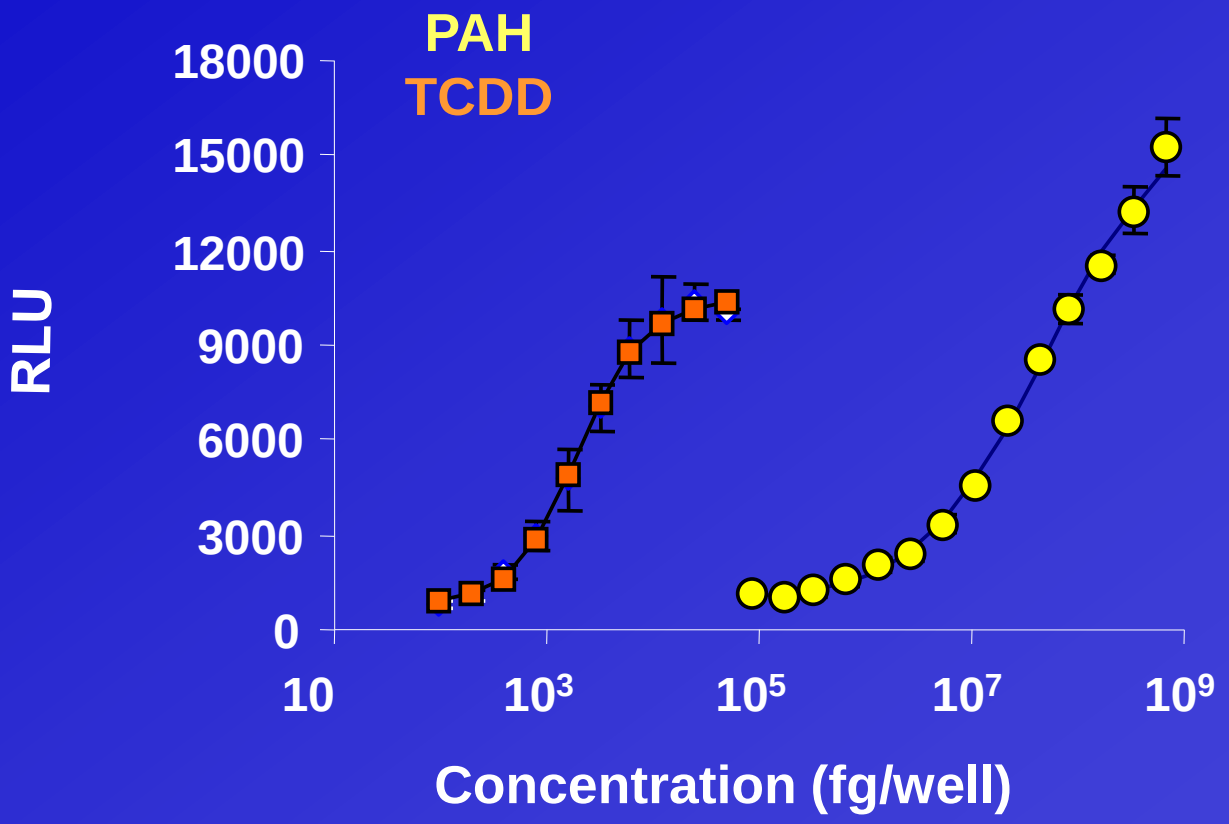


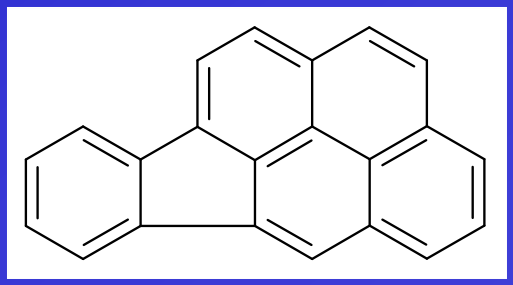
24 h exposure

Dose response curves

- *High response*

Indeno(1,2,3-c,d)pyrene

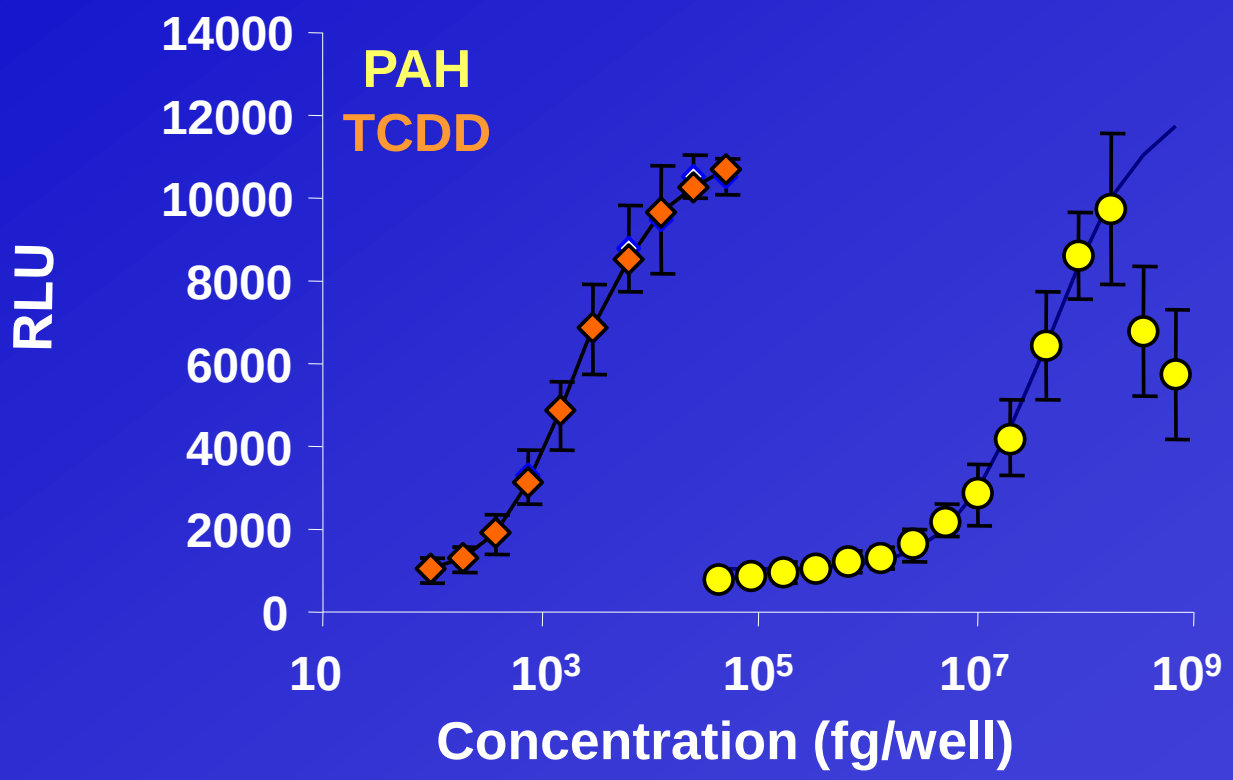




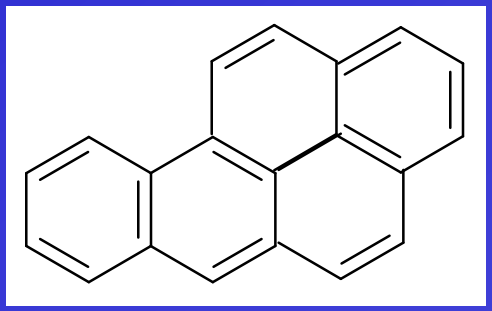
24 h exposure

Dose response curves

- *Decrease of response at high concentrations*



Benzo(a)pyrene



24 h exposure

REP values

- *Non-parallell dose response curves → responses from EC_{20} and EC_{80} to derive $REP_{20\text{-}80}$ range (Villeneuve et al., 2000)*

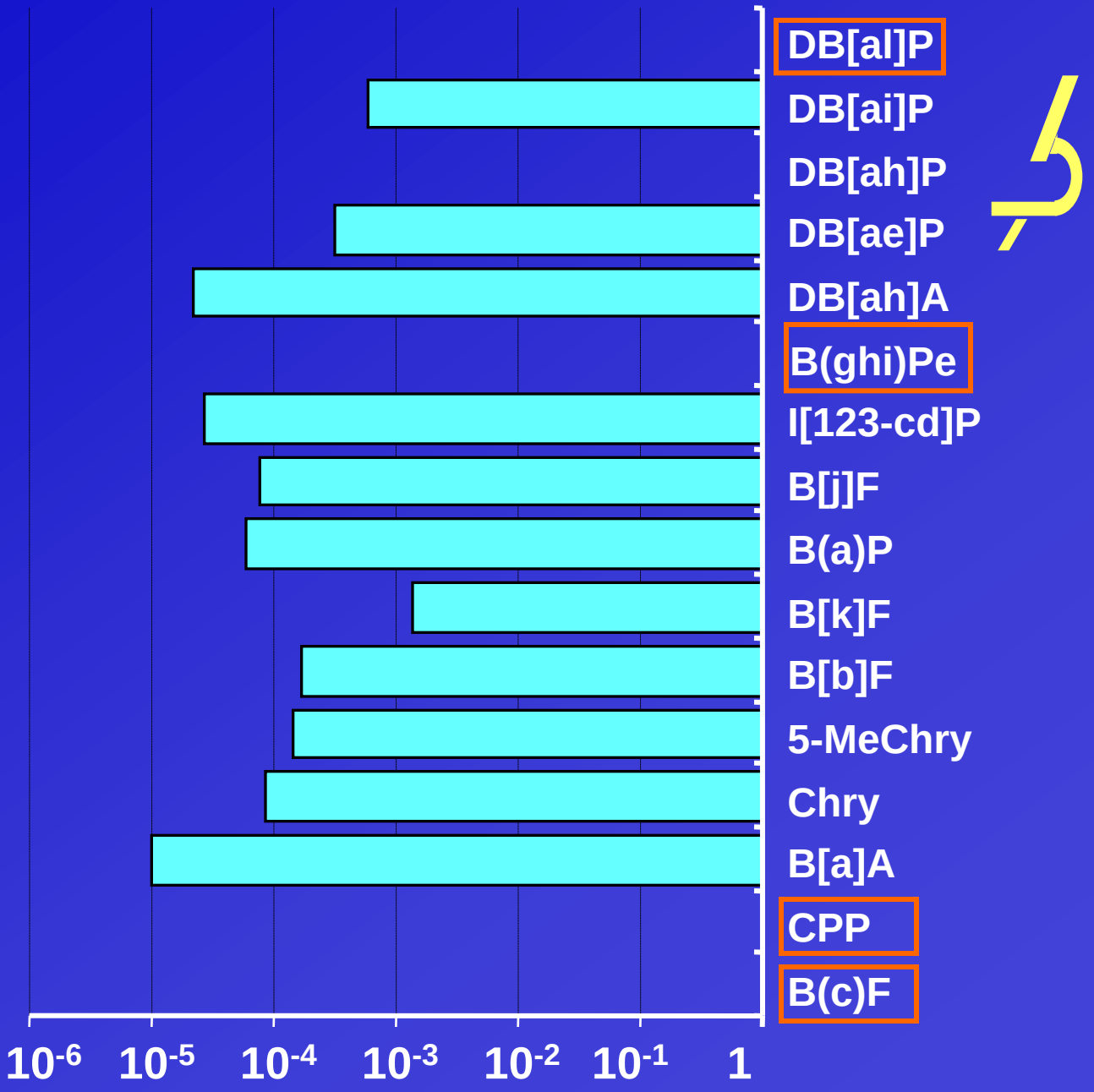
24 h exposure

REP-values

	CALUX REP weight	REP range EC_{20}-EC_{80}	CALUX REP molar	Efficacy % of TCDD
B[c]F	NI	NI	NI	9
CPP	NI	NI	NI	15
B[a]A	1 E-5 ± 2 E-6	8 E-5 - 3 E-6	7 E-6 ± 1 E-6	52
Chry	8 E-5 ± 6 E-6	4 E-4 - 8 E-5	6 E-5 ± 4 E-6	51
5MeChry	1 E-4 ± 1 E-5	3 E-4 - 1 E-4	1 E-4 ± 1 E-5	64
B[a]P	6 E-5 ± 9 E-6	6 E-5 - 6 E-5	5 E-5 ± 7 E-6	88
B[b]F	2 E-4 ± 2 E-5	4 E-4 - 1 E-4	1 E-4 ± 1 E-5	95
B[k]F	1 E-3 ± 2 E-4	4 E-3 - 1 E-3	1 E-3 ± 2 E-4	110
B[j]F	8 E-5 ± 1 E-5	2 E-4 – 6 E-5	6 E-5 ± 1 E-5	120
I[123-cd]P	3 E-5 ± 3 E-6	9 E-5 – 8 E-6	2 E-5 ± 3 E-6	143
B[ghi]Pe	NI	NI	NI	23
DB[ah]A	2 E-5 ± 3 E-6	4 E-4 – 2 E-6	2 E-5 ± 3 E-6	125
DB[ae]P	3 E-4 ± 3 E-5	5 E-4 – 4 E-4	3 E-4 ± 3 E-5	210
DB[ah]P	NC	NC	NC	117
DB[ai]P	6 E-4 ± 7 E-5	6 E-3 – 6 E-4	6 E-4 ± 6 E-5	116
DB[al]P	NI	NI	NI	24

relative to TCDD, 24 h exposure

REP values



Relative to TCDD, 24 h exposure; weight derived

Comparison with Toxicity equivalent Factors (TEF) (US-EPA, 1993)

Substance	TEF	REP
Benzo(a)pyrene	1	1
Dibenzo(a,h)antracene	1	*
benzo(a)antracene	0.1	0.2
benzo(b)fluoranthene	0.1	3
benzo(k)fluoranthene	0.1	22
Indeno(1,2,3-cd)pyrene	0.1	0.4
chrysene	0,01	1.4

Relative to TCDD, 24 h exposure

Comparison with literature data

PAH	This study	Ziccardi et al.(2000)	Ziccardi et al. (2002)	Clemons et al. (1998)
cell line	*H1L6.1*	*H1L1.1c2*	*H1L1.1c2*	*Hepa1c1c7*
B[c]F	NI	-	-	-
CPP	NI	-	-	-
B[a]A	7 E-6	2 E-3	NI	1 E-5
Chry	6 E-5	-	1 E-4	1 E-2
5Me-chry	1 E-4	-	-	-
B[a]P	5 E-5	4 E-3	8 E-5	1 E-5
B[b]F	1 E-4	-	2 E-4	-
B[k]F	1 E-3	4 E-1	2 E-2	5 E-2
B[j]F	6 E-5	-	-	-
I[123-cd]P	2 E-5	-	3 E-3	-
B[ghi]Pe	NI	-	NI	-
DB[ah]A	2 E-5	-	2 E-3	5 E-2
DB[ae]P	3 E-4	-		-
DB[ah]P	NC	-		-
DB[ai]P	6 E-4	-		-
DB[al]P	NI	-		-

Mouse cell lines

Conclusion (1)

- PAHs can significantly contribute to the TEQ-value determined by means of the 24 h CALUX bioassay.
- Nevertheless, the ranking of REP values for different PAHs obtained in the present study is comparable with the results from other researchers using an *in vitro* luciferase bioassay.
- Additionally, the CALUX response was investigated for the EU priority PAHs B[c]F and B[j]F, for which no REP-values were known until now.

European regulation on PAHs in food

- European regulation (EC 208/2005) on food contaminants:
  - Maximum levels for benzo(a)pyrene (BaP) in food
  - Use of BaP as a marker substance for all PAHs

→ better to express PAH toxicity in BaP-TEQ instead of TCDD-TEQ

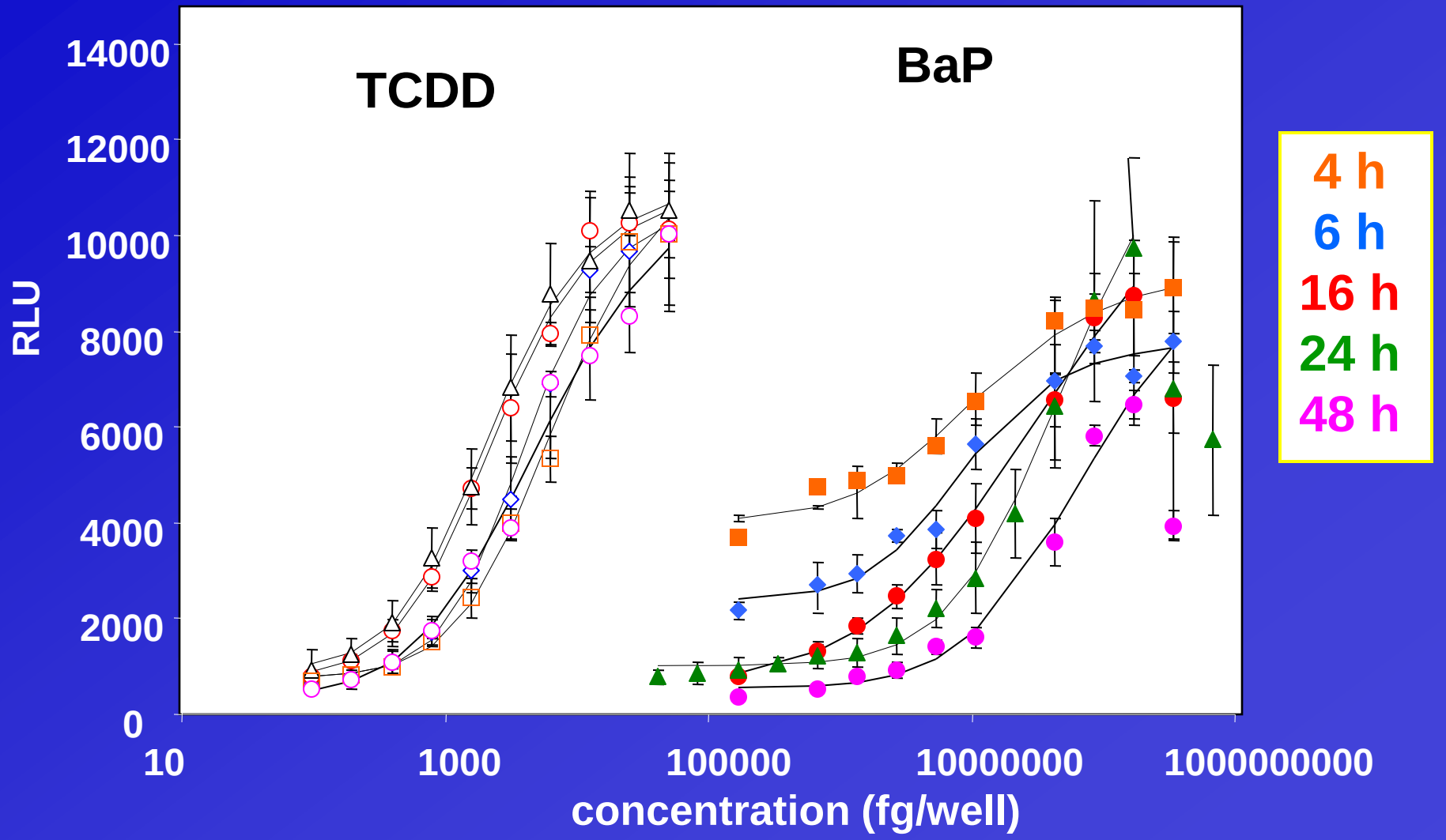
Use of the CALUX assay for PAHs

- Literature:
  - exposure times between 3h and 24h
  - Reference compound:
    - TCDD
    - Benzo(a)pyrene

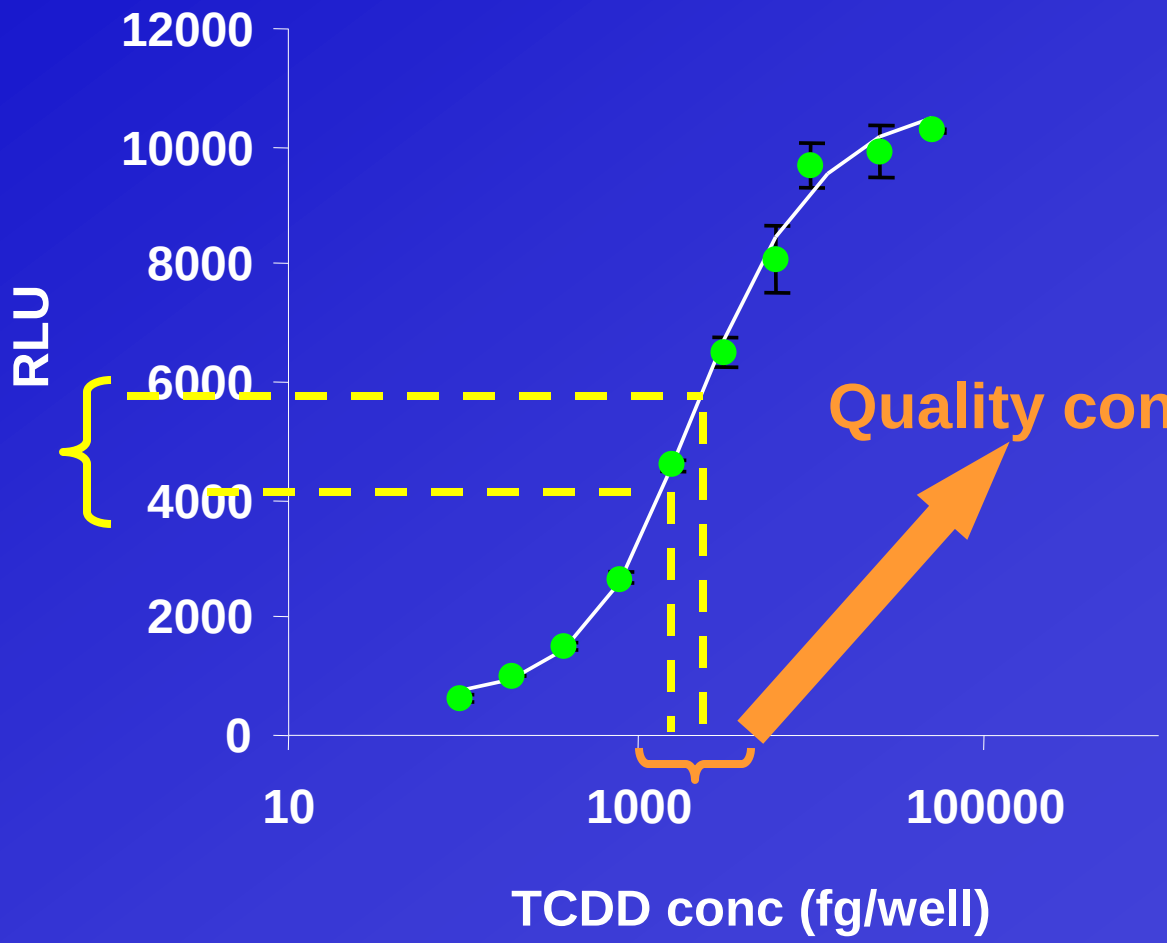
- Maximum induction of luciferase activity after 6-12 h (Postlind (1993), Machala et al. (2001); Villeneuve et al. (2002); Masunaga et al. (2004))

→ Metabolic decomposition of PAHs

Influence of exposure time

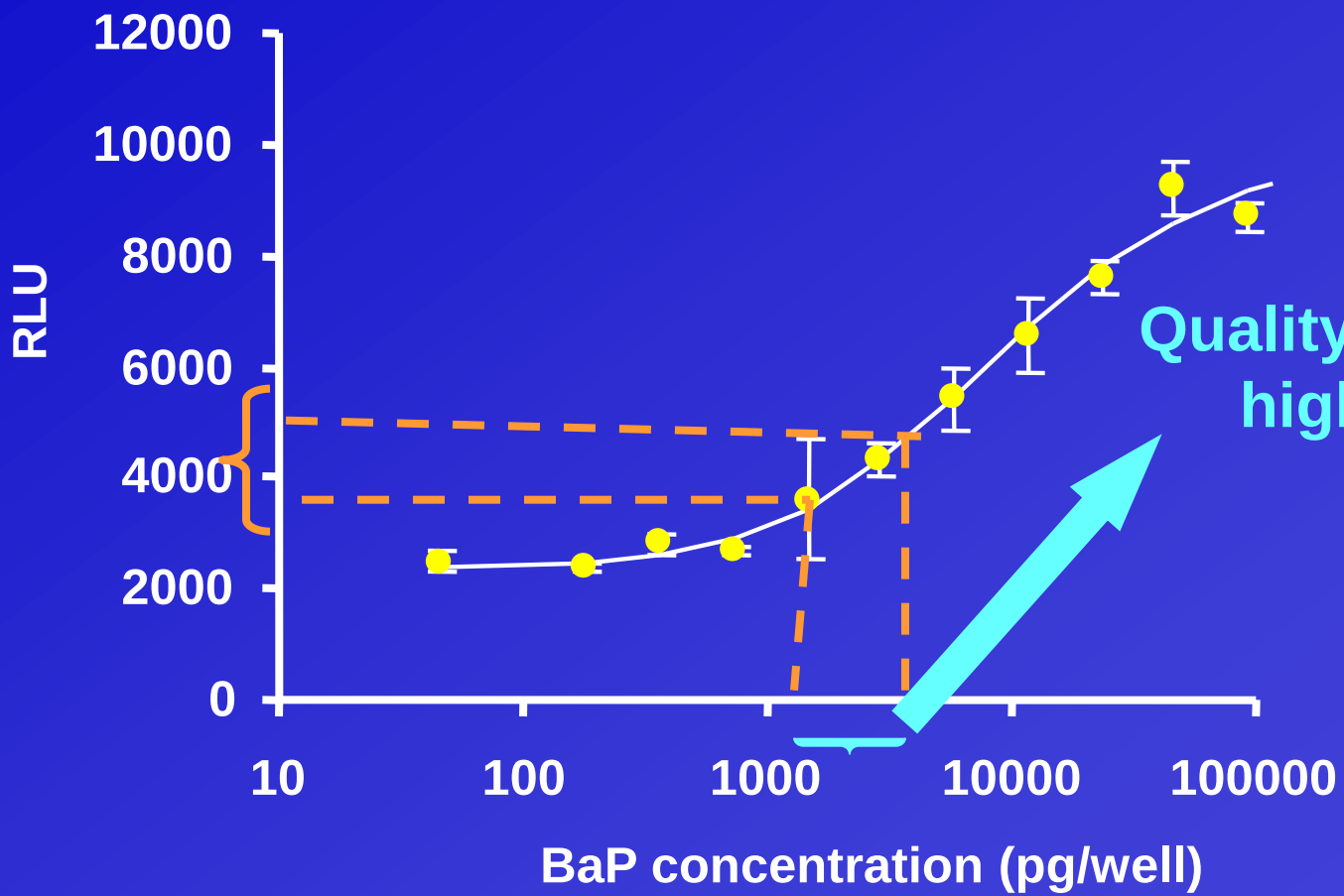


24 h exposure time, TCDD



Quality control: RSD< 15%

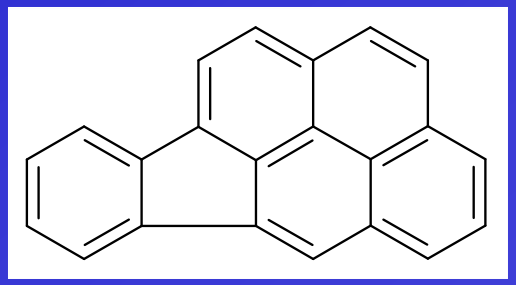
6 h exposure time, BaP

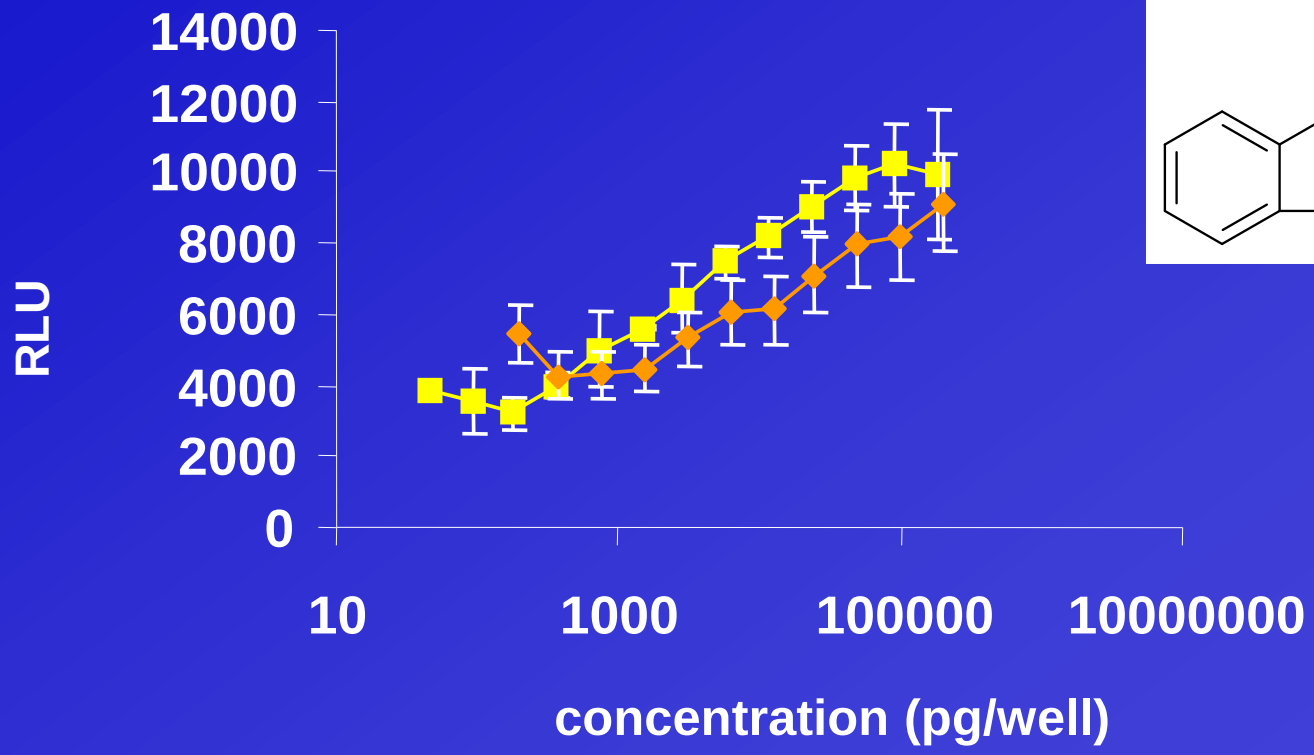


TEQ values

Dose response curves, 6 h exposure

Indeno(1,2,3-c,d)pyrene



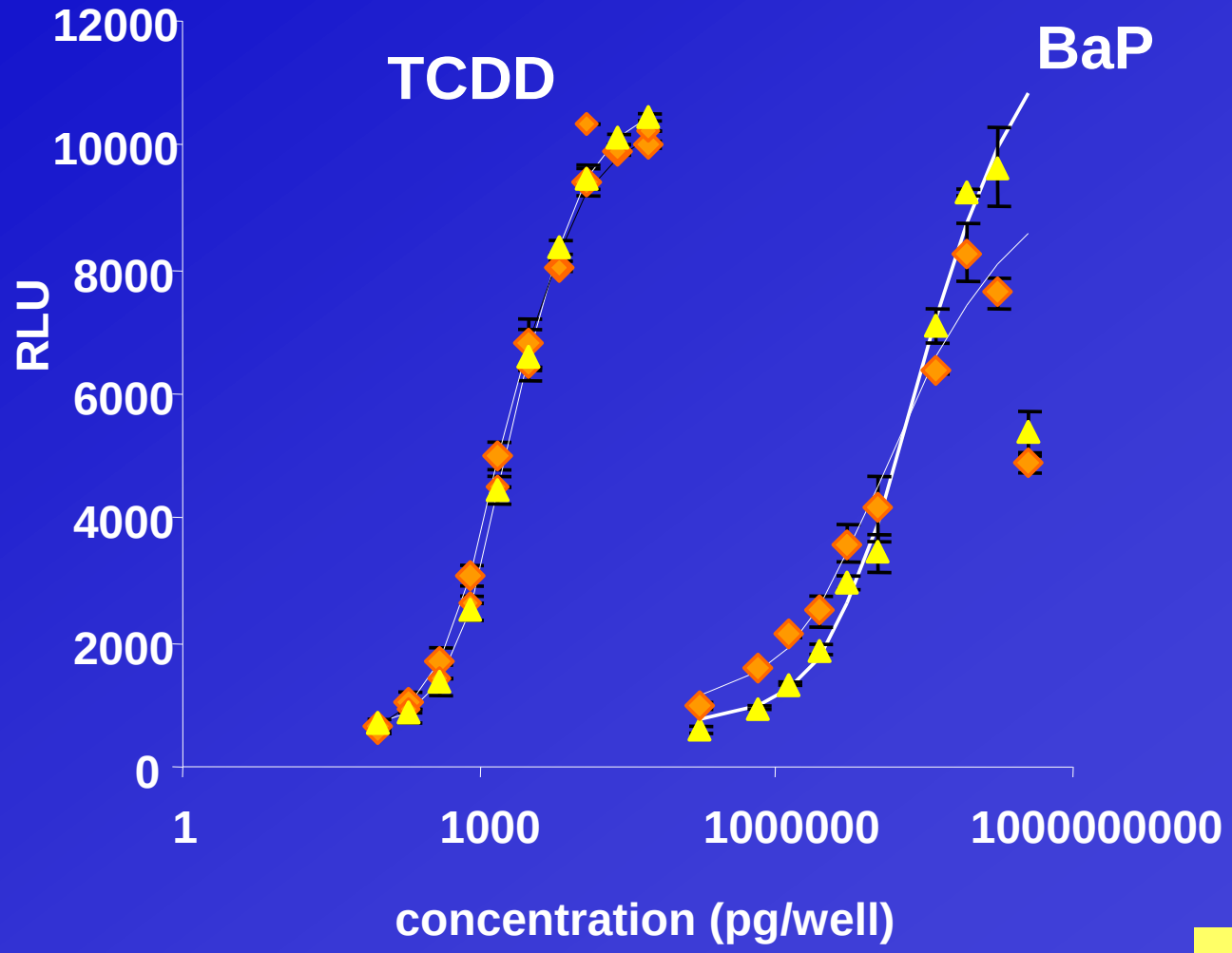


not always a sigmoidal shape

Conclusion (2)

- Response of PAH = f(exposure time)
- 6 h exposure: higher variability in response compared to 24 h incubation
- not always a sigmoidal shape of the curve (6 h exposure)
- RLU: RSD = 6-15%
  TEQ: much higher RSD because of ‘flat’ callibration curve
  => problem for quality control

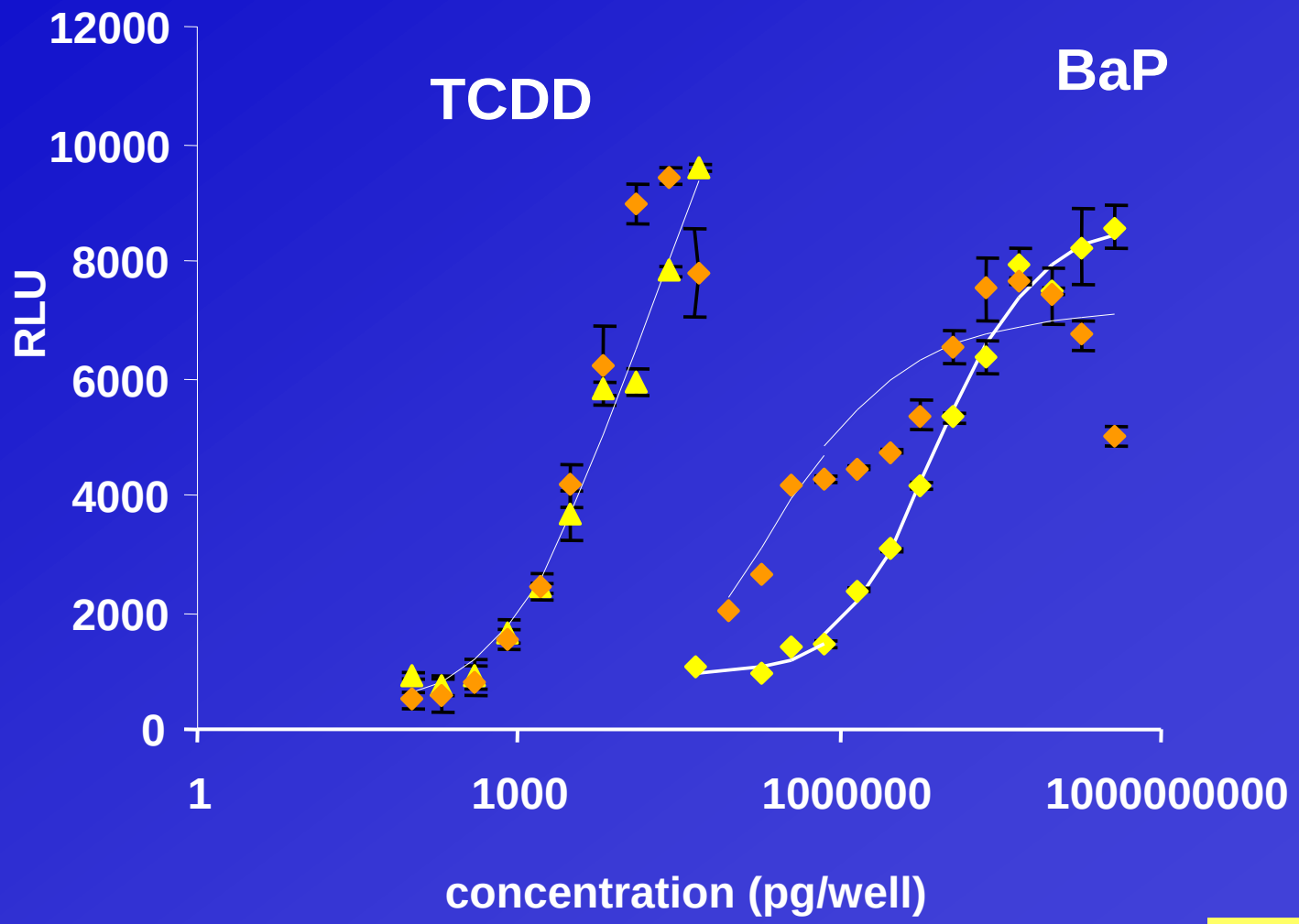
Influence of incubation temperature



37 °C
31 °C

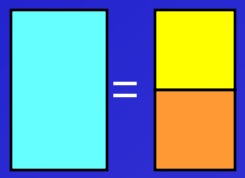
24 h exposure

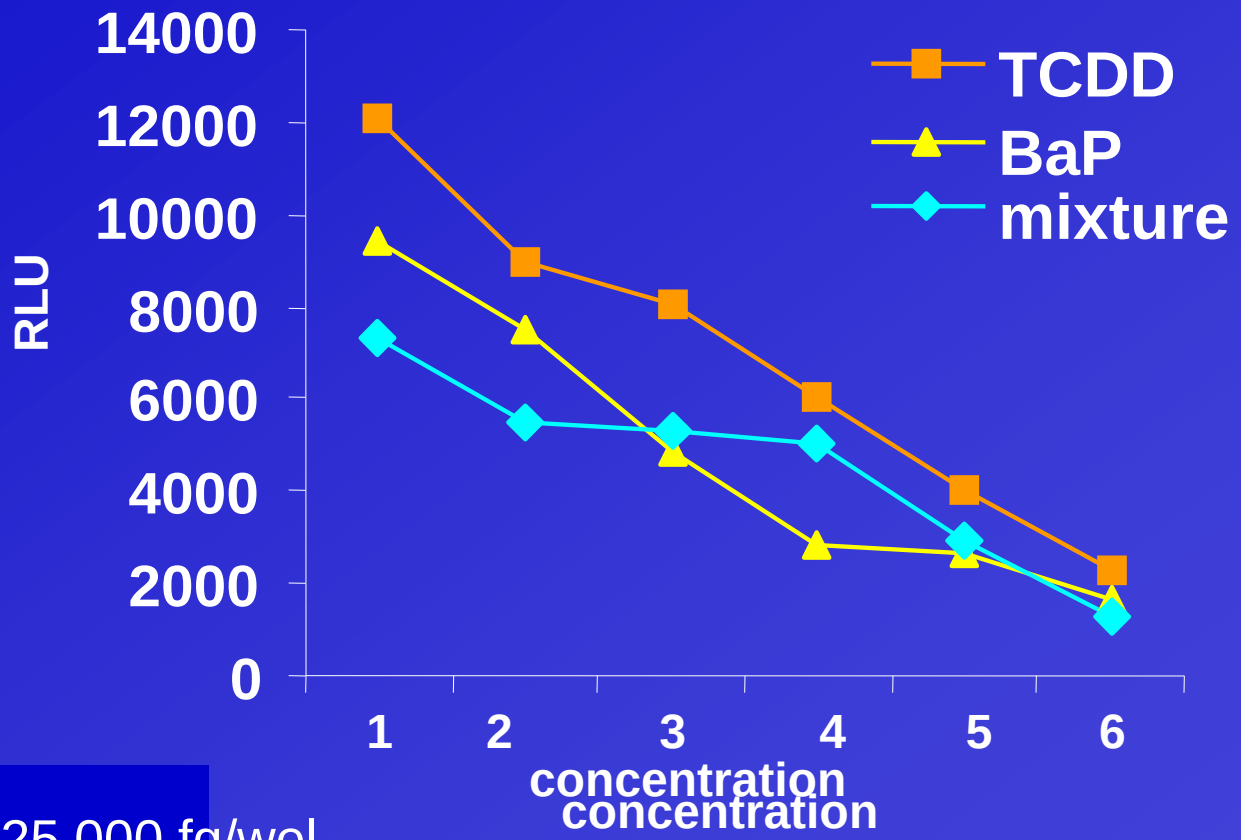
Influence of incubation temperature



6 h exposure

Antagonostic effects?





1: TCDD: 25 000 fg/wel
BaP: 93 000 pg/well

24 h exposure